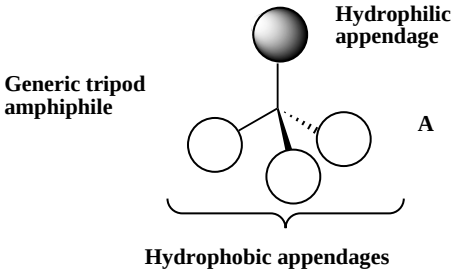
[4] a) A. V. Eliseev, M. I. Nelen, *J. Am. Chem. Soc.* **1997**, *119*, 1147 – 1148; b) A. V. Eliseev, M. I. Nelen, *Chem. Eur. J.* **1998**, *4*, 825 – 834.
[5] a) P. A. Brady, J. K. M. Sanders, *J. Chem. Soc. Perkin Trans. 1* **1997**, 3237 – 3253; b) H. Hioki, W. C. Still, *J. Org. Chem.* **1998**, *63*, 904 – 905; c) M. Albrecht, O. Bau, R. Fröhlich, *Chem. Eur. J.* **1999**, *5*, 48 – 56; d) I. Huc, M. J. Krische, D. P. Funeriu, J.-M. Lehn, *Eur. J. Inorg. Chem.* **1999**, 1415 – 1420.
[6] For recent overviews, see: a) A. Ganesan, *Angew. Chem.* **1998**, *110*, 2989 – 2992; *Angew. Chem. Int. Ed.* **1998**, *37*, 2828 – 2831, and references therein; b) C. Gennari, H. P. Nestler, U. Piarulli, B. Salom, *Liebigs Ann.* **1997**, 637 – 647, and references therein; c) J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455 – 2463.
[7] Several examples of host-templated selection of the strongest binder in a dynamic library of guest molecules were recently reported: a) I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106 – 2110; b) S. Sakai, Y. Shigemassa, T. Sasaki, *Tetrahedron Lett.* **1997**, *38*, 8145 – 8148; c) B. Klekota, M. H. Hammond, B. L. Miller, *Tetrahedron Lett.* **1997**, *38*, 8639 – 8642.
[8] a) S. J. Rowan, P. A. Brady, J. K. M. Sanders, *Angew. Chem.* **1996**, *108*, 2283 – 2285; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2143 – 2145; b) S. J. Rowan, J. K. M. Sanders, *Chem. Commun*. **1997**, 1407 – 1408; c) S. J. Rowan, P. A. Brady, J. K. M. Sanders, *J. Am. Chem. Soc*. **1997**, *119*, 2578 – 2579.
[9] a) R. H. Vreekamp, J. P. M. van Duynhoven, M. Hubert, W. Verboom, D. N. Reinhoudt, *Angew. Chem.* **1996**, *108*, 1306 – 1309; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1215 – 1218; b) P. Timmerman, R. H. Vreekamp, R. Hulst, W. Verboom, D. N. Reinhoudt, K. Rissanen, K. A. Udachin, J. Ripmeester, *Chem. Eur. J.* **1997**, *3*, 1823 – 1832; c) K. A. Jolliffe, M. Crego Calama, P. Timmerman, R. Fokkens, N. M. M. Nibbering, D. N. Reinhoudt, *Angew. Chem.* **1998**, *110*, 1294 – 1297; *Angew. Chem. Int. Ed.* **1998**, *37*, 1247 – 1251; d) L. J. Prins, J. Huskens, F. de Jong, P. Timmerman, D. N. Reinhoudt, *Nature* **1999**, *398*, 498 – 502.
[10] For a general overview of noncovalent synthesis, see: G. M. Whitesides, E. E. Simanek, J. P. Mathias, C. T. Seto, D. N. Chin, M. Mammen, D. M. Gordon, *Acc. Chem. Res.* **1995**, *28*, 37 – 44, and references therein.
[11] M. Crego Calama, R. Hulst, R. Fokkens, N. M. M. Nibbering, P. Timmerman, D. N. Reinhoudt, *Chem. Commun*. **1998**, 1021 – 1022.
[12] The number of assemblies (P) that are formed upon assembling N different components is $P = N^2 + \frac{1}{2}N(N-1)(N-2)$.
[13] Dimelamine **2** was synthesized in 60% yield (two steps) by the reaction of the bis(chlorotriazine) derivative **3** with (excess) 1,3-propane diamine followed by reaction with Zn-[5-(4-chlorocarbonylphenyl)-10,15,20-tris(4-methylphenyl)-porphyrin] (1.2 equiv).
[14] H. L. Anderson, J. K. M. Sanders, *J. Chem. Soc. Chem. Commun*. **1989**, 1714 – 1715.
[15] H. L. Anderson, J. K. M. Sanders, *Angew. Chem.* **1990**, *102*, 1478 – 1480; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 1400 – 1403.
[16] The $\delta = 13 - 15$ region of the ^{1}H NMR spectrum of the library is not very informative due to extensive overlap of signals for homomeric and heteromeric assemblies.
[17] The experimental distribution was determined by integration of the proton signals in the $\delta = 2.5 - 2.7$ region ($H_{f,g}$). The calculated distribution of 25/75% reflects the threefold higher probability for formation of the heteromeric assemblies.
[18] The additional small signals present in spectrum 4D ($\delta = 13 - 15$, around $\delta = 8.8$, and the two shoulders of the singlet at $\delta = 2.62$) are due to the presence of a slight excess of **4**, giving rise to the formation of small amounts of the heteromeric assembly $\mathbf{1b}_2 \cdot \mathbf{2} \cdot (\mathrm{DEB})_6 \cdot \mathbf{4}_2$. Evidence for this comes from the fact that the intensity of these signals increases when **4** is present in larger excess.
[19] P. Timmerman, D. N. Reinhoudt, *Adv. Mater.* **1999**, *11*, 71 – 74.

Rigid Amphiphiles for Membrane Protein Manipulation**

D. Tyler McQuade, Mariah A. Quinn, Seungju M. Yu, Arthur S. Polans, Mark P. Krebs,* and Samuel H. Gellman*

The shape of an amphiphile strongly influences self-association in solution[1] and in liquid crystalline phases,[2] as well as interactions with self-assembled structures such as lipid bilayers.[3] In recent years several groups have examined unusual amphiphile topologies.[4, 5] We and others, for example, have explored amphiphiles in which hydrophilic groups project on one side of an approximately planar hydrophobic unit ("contrafacial amphiphiles").[4] Here we introduce a related family of molecules based on a rigid quaternary carbon center, "tripod amphiphiles" (**A**), and present evidence that these amphiphiles can solubilize the two non-homologous membrane proteins bacteriorhodopsin (BR) and bovine rhodopsin (Rho) in a stable monomeric state.



Intrinsic membrane proteins perform many crucial functions, including transport, catalysis, photosynthesis, respiration, and signal transduction. The detailed study of membrane protein structure requires that the protein be isolated in a soluble native-like conformation, which in turn requires the use of a synthetic amphiphile (a detergent) to shield large

[*] Prof. S. H. Gellman, D. T. McQuade, M. A. Quinn, Dr. S. M. Yu
Department of Chemistry
University of Wisconsin
1101 University Ave., Madison, WI 53706 (USA)
Fax: (+1) 608-265-4534
E-mail: gellman@chem.wisc.edu
Prof. M. P. Krebs
Department of Biomolecular Chemistry
University of Wisconsin Medical School
1215 Linden Drive, Madison, WI 53706 (USA)
E-mail: mpkrebs@facstaff.wisc.edu
Prof. A. S. Polans
Department of Ophthalmology and Visual Sciences
University of Wisconsin Medical School
1215 Linden Drive, Madison, WI 53706 (USA)

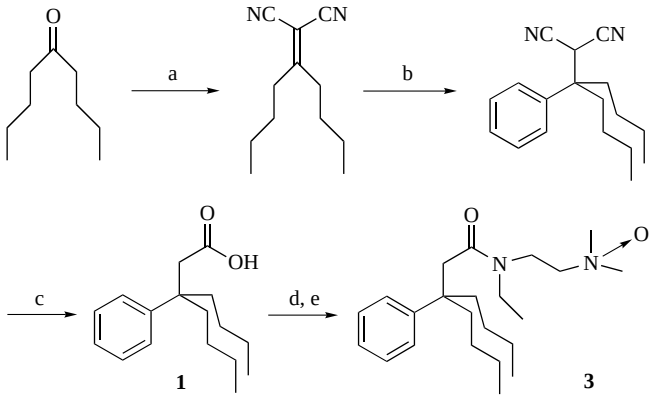
[**] We thank D. McCaslin for assistance with circular dichroism and analytical ultracentrifugation measurements, and M. Garavito for helpful suggestions. The work was funded by the NIH (R21 GM59351). Some of the measurements were conducted in the UW Biophysics Instrumentation Facility (NSF BIR-9512577). D.T.M. was supported in part by a Fellowship from the Organic Division of the American Chemical Society, sponsored by Pharmacia & Upjohn. M.A.Q. was a Hilldale Undergraduate Research Fellow. S.M.Y. was supported by a fellowship from the NIH.

  0570-0833/00/3904-0758 $ 17.50+.50/0

hydrophobic patches on the protein surface. X-ray crystallography is the best method for obtaining high-resolution structural information on membrane proteins; however, crystallizing membrane proteins has proven to be a significant obstacle to this form of analysis.[6] Thus, an amphiphile must perform two functions if it is to support crystallization: 1) solubilize the membrane protein in its native form for at least several weeks (long enough for the growth of high-quality crystals), and 2) contribute to the crystalline lattice stability.[7] Our results show that the first criterion can be fulfilled by a tripod amphiphile, which is an important step toward our long-term goal of using these molecules to facilitate the structural analysis of membrane proteins.

Our interest in tripod amphiphiles was motivated by the hypothesis that crystallization of complexes of membrane proteins and amphiphiles would be promoted by increasing amphiphile rigidity.[8] The detergents used for the crystallization of membrane proteins usually contain very flexible linear alkyl chains; these detergent molecules remain disordered even when high-quality crystals form.[7] Tripod amphiphiles are designed to be more conformationally rigid than conventional detergents while retaining the ability to bind to and shield the large hydrophobic surfaces displayed by membrane proteins.[6] Tripod amphiphiles are built around a quaternary carbon atom that bears one polar and three nonpolar appendages. The quaternary carbon atom introduces a substantial conformational restriction[9] relative to the linear alkyl chains common among detergents.

Our synthetic route to tripod amphiphiles is modular, as illustrated by the synthesis of acid **1** outlined in Scheme 1, which allows for the facile variation of the hydrophilic and

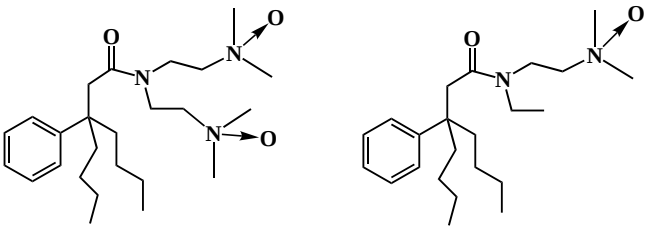


Scheme 1. Synthetic route leading to tripod amphiphile **3**: a) $CH_2(CN)_2$, AcOH, NH_4OAc, benzene, reflux; b) PhMgBr, CuI, diethyl ether, 0 °C; c) KOH, ethylene glycol, reflux; d) 1) oxalyl chloride, benzene, 0 °C; 2) *N*-ethyl-*N*′,*N*′-dimethylethylenediamine, diethyl ether, 0 °C; e) *m*CPBA, chloroform, −10 °C. *m*CPBA = *meta*-chloroperbenzoic acid.

hydrophobic regions.[10] The conformational flexibility of tripod amphiphiles can be tuned by the choice of hydrophobic appendages. This tunability is important, since a minimum level of amphiphile flexibility is thought to be crucial for membrane protein solubilization.[11] Conventional detergents used for membrane protein manipulation nearly all bear nonionic or zwitterionic hydrophilic moieties; we selected the N-oxide group, which could be readily introduced through derivatization of **1**, for our initial studies.

We utilized BR solubilization as an initial screen to identify promising tripod amphiphiles. BR is an excellent model system for evaluating membrane protein manipulation methods; several groups have used this protein for solubilization and crystallization studies.[12] BR assembles naturally into two-dimensional crystalline domains in the *Halobacterium salinarum* membrane, and this BR–lipid assembly ("purple membrane") is readily isolated. Four conventional detergents have been reported to solubilize BR from the purple membrane: Triton X-100, octylglucoside, nonylglucoside, and octylthioglucoside.[12] (Many other detergents denature BR.) Triton X-100 is the most effective solubilizer, but there are no reports of any membrane protein being crystallized in the presence of Triton X-100, perhaps because this detergent is heterogeneous and/or because the oligoether portion is highly flexible. Several additional amphiphiles, such as dodecylmaltoside, $C_{12}E_8$, reduced Triton X-100, "amphipols",[13] CHAPSO[14] (a zwitterionic cholate derivative), and a "peptitergent"[15] (a 24 residue peptide that adopts an amphiphilic α-helix), have been reported to maintain the solubility of BR after extraction from purple membrane with nonylglucoside or TX-100 followed by detergent exchange.[12] Recently, Landau and Rosenbush demonstrated that high-quality crystals of BR could be obtained by crystallization from a lipidic cubic phase. The crystals obtained provided a high-resolution, three-dimensional crystal structure (2.8 Å).[16]

Preliminary experiments suggested that bis-*N*-oxide **2** was able to solubilize BR from the purple membrane, but subsequent efforts revealed that very pure samples of **2** did not have this capacity. We discovered that heating of **2** during the initial preparation had inadvertantly induced a small proportion to undergo Cope elimination. Since the product after double Cope elimination (an *N*,*N*-divinyl amide) is probably not soluble in water, we speculated that the intermediate mono-*N*-oxide was the BR-solubilizing agent. We prepared **3**, a more accessible analogue of the monovinyl intermediate, to test this hypothesis. Tripod amphiphile **3** proved to be very effective at solubilizing BR from the purple membrane. Compound **3** is quite stable if stored at room temperature or below.

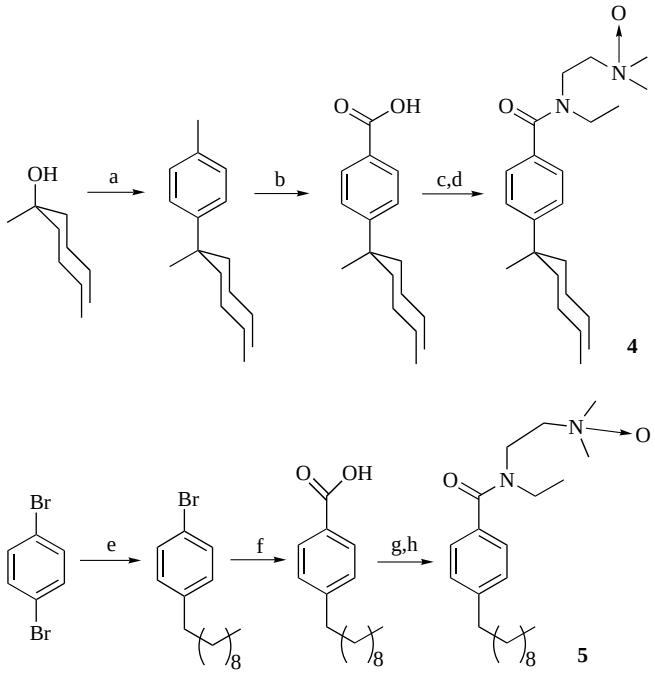


Solubilization was carried out by gently rocking a suspension of purple membrane in 25 mM aqueous phosphate buffer, pH 6.9, that contained 100 mM NaCl and tripod amphiphile **3**. The extent of BR solubilization was quantified by measuring the absorbance at 560 nm after the sample had been centrifuged to remove residual purple membrane and other insoluble material. Intact BR was extracted effectively from the purple membrane by solutions containing **3** at or above its

  0570-0833/00/3904-0759 $ 17.50+.50/0

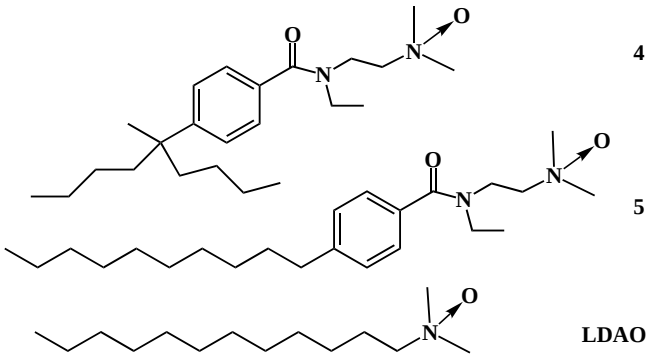
critical micelle concentration (estimated to be 3 mM by the 1,6-diphenylhexatriene solubilization method[17]). The rate of solubilization was evaluated by following the disruption of the purple membrane by circular dichroism. The close and regular spacing of BR molecules in the purple membrane lattice gives rise to exciton coupling between adjacent retinal Schiff base chromophores; this coupling is abolished when BR is solubilized.[18] Triton X-100 required more than 3 h for complete solubilization of BR, while solubilization by **3** was complete within 10 minutes. Analytical ultracentrifugation indicated that BR solubilized with 3 mM **3** (near the CMC) sediments as a dimer or higher oligomer, while BR solubilized with 6–12 mM **3** sediments as a monomer. BR solubilized with **3** shows little bleaching when stored at 4 °C (< 10 %) for 10 days.

We synthesized **4** and **5** (Scheme 2),[19] which are isomers of **3**, in order to probe the relationship between amphiphile structure and BR solubilization. These isomers represent an



Scheme 2. Synthesis of two constitutional isomers of **3**: a) $TiCl_4$, toluene, RT; b) hydroxypthalimide, $[Co(acac)_2]$, O_2, AcOH, RT; c) 1) oxalyl chloride, cat. DMF, benzene, 0 °C; 2) *N*-ethyl-*N*′,*N*′-dimethylethylenediamine, diethyl ether, 0 °C; d) *m*CPBA, chloroform, −10 °C; e) $[PdCl_2(dppf)]$, *n*-decylMgBr, THF/diethyl ether; f) 1) *t*BuLi, diethyl ether, −78 °C; 2) dry CO_2; g) 1) oxalyl chloride, benzene, 0 °C; 2) *N*-ethyl-*N*′,*N*′-dimethylethylenediamine, diethyl ether, 0 °C; h) *m*CPBA, chloroform, −10 °C.

incremental transformation of the tripod amphiphile architecture into a more conventional detergent architecture, similar to that of lauryldimethylamine oxide (LDAO) with a polar N-oxide group at one end of a linear hydrophobic chain. Amphiphile **4** has a CMC of approximately 0.7 mM, and **5** has a CMC of about 0.1 mM (a CMC of about 2 mM has been reported for LDAO[20]). Like LDAO, **4** and **5** denature BR when exposed to purple membrane preparations. Thus, within the isomer series **3**–**5**, the tripod amphiphile architecture of **3** confers unique behavior with regard to BR.



The promising behavior of **3** toward BR prompted us to examine the solubilization of Rho with the amphiphile. Rho has been a subject of widespread interest because this protein is a G-protein-coupled receptor and therefore represents a large class of important signaling proteins.[21] The behavior of Rho with conventional detergents has been extensively studied, but no Rho–detergent complex has yet provided high quality three-dimensional crystals.[22] Only low-resolution structural information is presently available for Rho, which was obtained from two-dimensional crystals.[23]

Solubilization of Rho was carried out using rod outer segments (ROS) purified from bovine retina.[24] A mixture of ROS and 30 mM **3** was incubated for 30 min in 10 mM acetate buffer, pH 6.9, at room temperature. After centrifugation to remove membrane fragments, the supernatant was found to contain 90 % intact Rho, as judged by an absorbance at 500 nm.[25] Rho solubilized by **3** showed less than 10 % bleaching after storage for 20 days at 4 °C in the dark.

These results indicate that we have accomplished an important first step toward creating amphiphiles that can be used to crystallize membrane proteins. Growth of high-quality crystals is widely acknowledged to be the bottleneck in the structural analysis of intrinsic membrane proteins.[6] Low molecular weight amphiphiles are essential for crystallization, but relatively little effort has been devoted to the synthesis and evaluation of alternatives to commercially available detergents. Since the tripod skeleton is easily modified, this new class of amphiphiles could prove to be very useful for membrane protein manipulation. We are currently initiating collaborative crystallization efforts.[26]

Received: September 6, 1999 [Z 13971]

[1] J. Israelachvili, *Intermolecular & Surface Forces*, 2nd ed., Academic Press, New York, **1991**.
[2] a) E. E. Burnell, C. A. de Lange, *Chem. Rev.* **1998**, *98*, 2359; b) C. Tschierske, *J. Mater. Chem.* **1998**, *8*, 1485; c) J. S. Moore, *Curr. Opin. Solid State Mater. Sci.* **1996**, *1*, 777.
[3] K. Nada, A. Sadownik, S. L. Regen, *J. Am. Chem. Soc.* **1993**, *115*, 2278.
[4] a) D. T. McQuade, D. G. Barrett, J. M. Desper, R. Hayashi, S. H. Gellman, *J. Am. Chem. Soc.* **1995**, *117*, 4862; b) D. G. Barrett, S. H. Gellman, *J. Am. Chem. Soc.* **1993**, *115*, 9343; c) T. M. Stein, S. H. Gellman, *J. Am. Chem. Soc.* **1992**, *114*, 7319; d) Y. Cheng, D. M. Ho, C. R. Gottlieb, D. Kahne, M. A. Bruck, *J. Am. Chem. Soc.* **1992**, *114*, 7319; e) P. Venkatesan, Y. Cheng, D. J. Kahne, *J. Am. Chem. Soc.* **1994**, *116*, 6955; f) V. Janout, M. Lanier, S. L. Regen, *J. Am. Chem. Soc.* **1997**, *119*, 640.

  0570-0833/00/3904-0760 $ 17.50+.50/0

[5] a) M. Kolbel, T. Beyersdorff, I. Sletvold, C. Tschierske, J. Kain, S. Diele, *Angew. Chem.* **1999**, *111*, 1146; *Angew. Chem. Int. Ed.* **1999**, *38*, 1077; b) V. Sidorov, T. Douglas, S. M. Dzekunov, D. Abdallah, B. Ghebremariam, P. D. Roepe, S. Matile, *Chem. Commun.* **1999**, 1429; c) J. H. Fuhrhop, D. Fritsch, *Acc. Chem. Res.* **1986**, *19*, 130; d) R. Zana, *Curr. Opin. Coll. Inter. Sci.* **1996**, *1*, 566.

[6] Selected reviews: a) C. Ostermeier, H. Michel, *Curr. Opin. Struct. Biol.* **1997**, *7*, 697; b) R. M. Garavito, D. Picot, P. J. Loll, *J. Bioenerg. Biomembr.* **1996**, *28*, 13; c) J. M. Sowadski, *Curr. Opin. Struct. Biol.* **1994**, *4*, 761; d) H. Michel, *Crystallization of Membrane Proteins*, CRC, Boca Raton, **1990**; e) W. Kühlbrandt, *Q. Rev. Biophys.* **1988**, *21*, 429.

[7] The structure of detergents have been difficult to analyze in membrane protein crystals, because of extensive disorder. Only a handful of such studies have been reported: a) M. Roth, L.-A. Bentley, H. Michel, J. Deisenhofer, R. Huber, D. Oesterhelt, *Nature* **1989**, *340*, 659; b) M. Roth, B. Arnoux, A. Ducruix, A. Reiss-Husson, *Biochemistry* **1991**, *30*, 9403; c) P. Timmins, E. Pebay-Peyroula, W. Welte, *Biophys. Chem.* **1994**, *53*, 27; d) E. Pebay-Peyroula, R. M. Garavito, J. P. Rosenbusch, M. Zulauf, P. A. Timmins, *Structure* **1995**, *3*, 1051.

[8] For a discussion of the inluence of a rigid amphiphile on the photostate of Rho, see A. Schleicher, R. Franke, K. P. Hofmann, H. Finkelmann, W. Welte, *Biochemistry* **1987**, *26*, 5908.

[9] a) R. W. Hoffmann, M. Stahl, U. Schopfer, G. Frenking, *Chem. Eur. J.* **1998**, *4*, 559; b) R. W. Hoffmann, *Angew. Chem.* **1992**, *104*, 1147; *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1124; c) R. W. Alder, C. M. Maunder, A. G. Orpen, *Tetrahedron Lett.* **1990**, *31*, 6717.

[10] Dicyanoalkylidene synthesis: a) A. C. Cope, C. M. Hofmann, C. Wyckoff, E. Hardenbergy, *J. Am. Chem. Soc.* **1941**, *63*, 3452; b) E. Campaigne, D. Mais, E. M. Yokley, *Synth. Commun.* **1974**, *4*, 379; c) Copper-mediated grignard: N. Rabjohn, L. V. Phillips, R. J. Defeo, *J. Org. Chem.* **1959**, *24*, 1964; d) Functionalization of the dicyanide (not shown): C. Malet, O. Hindsgaul, *J. Org. Chem.* **1993**, *61*, 4649; e) Hydrolysis of the dicyanide: S. Patai, S. Dayagi, *J. Chem. Soc.* **1962**, 716.

[11] a) A. Helenius, D. R. McCaslin, E. Fries, C. Tanford, *Methods Enzymol.* **1979**, *56*, 734; b) L. M. Hjelmeland, A. Chrambach, *Methods Enzymol.* **1984**, *104*, 305.

[12] a) N. A. Dencher, M. P. Heyn, *Methods Enzymol.* **1982**, *88*, 5; b) H. Michel, *EMBO J.* **1982**, *1*, 1267; c) L. J. W. Miercke, P. E. Ross, R. M. Stroud, E. A. Dratz, *J. Biol. Chem.* **1989**, *264*, 7531; d) S. J. Milder, T. E. Thorgeirsson, L. J. W. Miercke, R. M.Stroud, D. S. Kliger, *Biochemistry* **1991**, *30*, 1751; e) G. F. X. Schertler, H. D. Bartunik, H. Michel, D. Oesterhelt, *J. Mol. Biol.* **1993**, *234*, 156.

[13] C. Tribet, R. Audebert, J.-L. Popot, *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 15047 – 15050.

[14] L. M. Hjelmeland, D. W. Nebert, J. C. Osborne, *Anal. Biochem.* **1983**, *130*, 72.

[15] C. E. Schafmeister, L. J. W. Miercke, R. M. Stroud, *Science* **1993**, *262*, 734.

[16] For the initial crystallization, see a) E. M. Landau, J. P. Rosenbusch, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 14532; b) For the crystal structure, see E. Pebay-Peyroula, G. Rummel, J. P. Rosenbusch, E. M. Landau, *Science* **1997**, *277*, 1676; c) For a refinement of the structure, see H. Luecke, H.-T. Richter, J. K.Lanyi, *Science* **1998**, *280*, 1934.

[17] A. Chattopadhyay, E. London, *Anal. Biochem.* **1984**, *139*, 408.

[18] a) J. Y. Cassium, *Biophys. J.* **1992**, *63*, 1432; b) T. G. Ebrey, B. Becher, B. Mao, P. Kilbride, B. Honig, *J. Mol. Biol.* **1977**, *112*, 377.

[19] a) Palladium-catalyzed aryl coupling: N. A. Bumagin, E. V. Luzikova, I. P. Beletskaya, *Russ. J. Org. Chem.* **1995**, *31*, 1480; b) Cobalt autoxidation: Y. Ishii, T. Iwahama, S. Sakaguchi, K. Nakayama, Y. Nishsiyama, *J. Org. Chem.* **1996**, *61*, 4520; c) aryl alkylation with titanium tetrachloride: N. M. Cullinane, D. M. Leyshon, *J. Chem. Soc.* **1954**, 2942.

[20] K. W. Herrmann, *J. Phys. Chem.* **1962**, *66*, 295.

[21] P. A. Hargrave, *Behav. Brain. Sci.* **1995**, *18*, 403.

[22] a) D. R. McCaslin, C. Tanford, *Biochemistry* **1981**, *20*, 5212; b) W. J. de Grip, *Methods Enzymol.* **1982**, *81*, 256; c) A. Krebs, C. Villa, P. C. Edwards, G. F. X. Schertler, *J. Mol. Biol.* **1998**, *282*, 991; d) W. J. de Grip, J. van Oostrum, G. L. J. de Caluwe, *J. Cryst. Growth* **1992**, *122*, 375.

[23] V. M. Unger, P. A. Hargrave, J. M. Baldwin, G. F. X. Schertler, *Nature* **1997**, *389*, 203.

[24] D. S. Papermaster, *Methods Enzymol.* **1982**, *81*, 48.

[25] The total amount of Rho present was estimated from the difference in the spectral absorbance at 500 nm before and after bleaching for 15 min under a white light.

[26] Preliminary studies (R. MacKinnon, personal communication) with a tripod amphiphile closely related to **3**, but with a modified N-oxide hydrophilic portion, reveal that the K^+ channel from *Streptomyces lividans* can be crystallized in the presence of this tripod amphiphile. These crystals, however, do not diffract X-rays as well as the crystals used for the published 3.2 Å-resolution structure of this protein (D. A. Doyle, J. M. Cabral, R. A. Pruetzner, A. L. Kuo, J. M. Gulbis, S. L. Cohen, B. T. Chait, R. Mackinnon, *Science* **1998**, *280*, 69), which were grown with LDAO.

The Total Synthesis of Frondosin B**

Masayuki Inoue, Alison J. Frontier, and Samuel J. Danishefsky*

Interleukin-8 (IL-8), a chemoattractant for neutrophils, is produced by macrophages and endothelial cells.[1] IL-8 promotes the accumulation and activation of neutrophils and has been implicated in a wide range of acute and chronic inflammatory disorders. Accordingly, blockade of IL-8-mediated chemotaxis represents a possible opportunity for the development of novel pharmacological agents. Frondosins A-E were recently isolated from the sponge *Dysidea frondosa* (Scheme 1). These compounds, which bear a casual relationship to one another, inhibit the binding of IL-8 to its receptor in the low micromolar range.[2a] The structures and relative stereochemistries of the frondosins were determined primarily by NMR spectroscopy. Their unifying feature is the presence of a bicyclo[5.4.0] ring system attached to variously permuted hydroquinione moieties.[3] A team from the National Cancer Institute (NCI) also isolated frondosins A and D from the HIV-inhibitory organic extract of the marine sponge.[2b, 4] It is worth noting that these compounds have optical rotations with different but opposite absolute values. Thus, the frondosins may occur as scalemic mixtures. Further biological studies of the frondosins could spur fruitful

[*] Prof. S. J. Danishefsky, Dr. M. Inoue
The Laboratory for Bioorganic Chemistry
Sloan-Kettering Institute for Cancer Research
1275 York Avenue, New York, NY 10021 (USA)
Fax: (+1) 212-772-8691
E-mail: s-danishefsky@ski.mskcc.org

Prof. S. J. Danishefsky, A. J. Frontier
The Department of Chemistry
Columbia University, New York, NY 10027 (USA)

[**] This work was supported by the National Institutes of Health (Grant Numbers: AI16943 and CA08748). M.I. gratefully acknowledges the Uehara Memorial Foudation for a postdoctoral fellowship. A.J.F. gratefully acknowledges the American Chemical Society Organic Division and Merck & Co. for Graduate Fellowship support. We thank Dr. George Sukenick of the MSKCC NMR Core Facility and Vinka Parmakovic of the Columbia University Mass Spectral Facility for mass spectral analyses.